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Small Ruminant Research 31 (1999) 203–208

Small Ruminant Research

Prevalence of subclinical intramammary infection caused by *Staphylococcus epidermidis* in a commercial dairy goat herd

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Accepted 26 May 1998

Abstract

One herd with 138 lactating goats that had a high somatic cell count (SCC) in bulk tank milk was studied. Foremilk was sampled for bacteriology and SCC at two samplings 28 days apart on the Dairy Herd Improvement Association test day. Blood samples were obtained for serological analyses of caprine arthritis–encephalitis virus. Bacterial intramammary infection (IMI) was diagnosed when the same pathogen was isolated twice from the same udder half. The prevalence of IMI was 34%. Most of the pathogens isolated (95.7%) were *Staphylococcus* spp. *Staphylococcus epidermidis* was the predominant species (66.7%), and most of these had similar biochemical profiles. Seroprevalence of caprine arthritis–encephalitis virus infection as assayed by agar gel immunodiffusion was 94.3%. Composite SCC as compiled by the Dairy Herd Improvement Association averaged 10^6/ml for the first sampling and 1.27×10^6/ml for the second sampling. Uninfected right and left udder halves had a lower foremilk SCC (1.3 and 1.0×10^6/ml, respectively) than the infected right and left udder halves (1.74 and 1.66×10^6/ml), respectively, but only in the left halves was the difference significant. Halves infected by *S. epidermidis* averaged higher SCC (1.8×10^6/ml) than the halves infected by other staphylococci (1.5×10^6/ml). Milk SCC increased as parity increased.

Keywords: Dairy goats; *Staphylococcus epidermidis*; Somatic cell count; Caprine arthritis–encephalitis virus

1. Introduction

The current legal limit for somatic cells in the bulk tank milk of goats in the US is 10^6/ml. Dairy goat farmers often experience difficulty in maintaining SCC below this threshold. Droke et al. (1993) evaluated SCC in bulk tank milk from 71 goat dairies located in Arkansas, Michigan, Wisconsin, and California. These researchers reported that 62% of the dairies had SCC $>10^6$/ml. High SCC are associated with IMI, but other factors such as stage of lactation, parity, and infection by caprine arthritis–encephalitis virus (CAEV), also contribute to elevated SCC (Dulin et al., 1983; Langlois et al., 1984; Poutrel, 1984; Smith and Cutlip, 1988). Wilson et al. (1995) reported that milk from infected and uninfected udder halves of goats frequently contain $>10^6$ somatic cells/ml. More than 90% of the variation in SCC was not due to bacterial IMI. Dulin et al. (1983) concluded that milk from uninfected goats could contain as many as 5×10^6 somatic cells/ml. Results of this study suggest that the

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0921-4488/99/$ – see front matter
PII: S0921-4488(98)00147-3

current legal limit for SCC of 10^6/ml may not be appropriate and could hinder development of the dairy goat industry in the US. There is no current legal limit in the European Union.

This study was designed to study factors involved in SCC in a commercial dairy goat herd in Maryland with a history of high SCC in bulk tank milk.

2. Materials and methods

2.1. Goats

A commercial dairy goat farm in southern Maryland with 138 lactating goats was studied. Goats were raised in an open herd, which was enrolled in the DHIA program. Laboratory analyses were conducted at the Mideast DHIA Laboratory (Hagerstown, MD), and records were processed at DHIA (Provo, UT). Monthly bulk tank SCC had exceeded 10^6/ml for 8 of the last 12 months. The herd had bacteriological survey results from 1988–1993 provided by the Animal Health Laboratory, Maryland Department of Agriculture (College Park). Coagulase-negative staphylococci (CNS) were the most prevalent organisms isolated. *Staphylococcus epidermidis* accounted for 30% of the isolates, and major pathogens, such as *Staphylococcus aureus, Streptococci* spp., or Gram-negative bacilli, were isolated infrequently. Prevalence of acute clinical mastitis in the last 10 year ranged from 0% to 2% clinical cases/year. Several clinical cases of CAEV had been observed. Does were of the Alpine (75%), Saanen (20%), and Nubian (5%) breeds. Data regarding production, age, parity, and day of lactation were obtained from the Mideast DHIA (Table 1). Does were machine-milked twice daily (12 h interval). Before milking, teats were sprayed with a sanitizing teat dip product containing 0.5% hydrogen peroxide, 1.7% lactic acid, and 0.5% sodium linear alkylate sulfonate (Oxy Gard®; Klenzade, St. Paul, MN). After this, each udder was dried with an individual disposable paper towel. After milking, teats were sprayed with the same product. At dry-off, all udder halves were infused with 300 mg of cephapirin benzathine (Tomorrow®; Franklin Laboratories, Fort Dodge, IA).

2.2. Sample collection

Duplicate foremilk samples were obtained twice (28 days interval) during October and November on the DHIA test day. Seven goats were sampled only once. Teats were carefully cleaned with 70% ethanol before sampling. The first three streams of foremilk were discarded, and the next 10 ml of milk were collected aseptically from each gland into sterile vials. Samples were kept at 4°C overnight until bacteriological procedures and SCC testing were performed. Composite bucket milk samples for the determination of SCC were obtained on the DHIA test day. Jugular blood samples were obtained from 88 does for analysis of antibodies to CAEV.

2.3. Bacteriological analyses

Twenty microliters of milk were spread onto the surface of a trypticase soy agar plate containing 0.1% esculin and 5% washed sheep erythrocytes (Remel, Lenexa, KS). The plates were incubated aerobically at 37°C and examined after 24 and 48 h. An infection was defined as >100 cfu/ml (2 colonies/plate) of the same organism. Cultures with two or more identical

Table 1
Age, parity, day of lactation, milk yield and composition for does studied

Variable	Number of goats with available data	Mean	SD	Minimum	Maximum
Age, year	135	3.1	1.7	1	9
Parity	137	2.3	1.6	1	9
Day of lactation	133	287	201	24	1173
Total milk yield, kg	133	933	760	554	5054
305-day milk yield, kg	126	1.136	400	554	3654
305-day fat yield, kg	126	41	29	17	319
305-day protein yield, kg	124	33	10	18	102

colonies were classified as positive. A true infection was defined as recovery of the same pathogen at either 24 or 48 h during both of the single samplings from each month (Andrews et al., 1983). Bacterial groups were identified according to the recommendations of the National Mastitis Council (Harmon et al., 1990). Specific identification of staphylococci was made using commercial micromethods (API STAPH®; bioMérieux Vitek, Hazelwood, MO).

2.4. Milk somatic cell counts

Samples were heated at 60°C for 15 min and maintained at 40°C until counted (Fossomatic 90; Foss Electric, Hillerϕd, Denmark). The cell counter was calibrated monthly with bovine milk somatic cell standards (Dairy Quality Control Institute Services, Mountain View, MN). Determination of composite milk SCC were performed by the Mideast DHIA.

2.5. Determination of antibodies to caprine arthritis–encephalitis virus

Serology determinations were performed by the Animal Health Diagnostic Laboratory (College Park, MD). Reactions were performed by agar gel immunodiffusion using the caprine arthritis–encephalitis and ovine progressive pneumonia antibody test kit (Veterinary Diagnostic Technology, Wheat Ridge, CO). The plates were examined after 24 h at 25°C and then again after 24 h at 2°C. Reactions were scored as negative, weak positive, and positive.

2.6. Analysis of results

Data were analyzed using the least-squares ANOVA procedures of SAS (SAS, 1996; Cary, NC). The model included effects of bacteriological status (infected or uninfected), parity (1, 2, or $\geq$3), and number of days in milk. Separate analyses were done for bacteriological status of left and right udder halves. For the purposes of analysis, all SCC were converted to natural logarithm scale.

3. Results

Of the 538 milk samples cultured for diagnostic bacteriology, 121 were positive (39.7%). Most of the isolates (95.7%) were *Staphylococcus* spp. Other bacteria isolated were *Streptococcus* spp. (1.4%), *Corynebacterium* spp. (1.4%), *Enterobacteriaceae* (0.9%), and *Bacillus* spp. (0.5%).

Ninety of the 262 udder halves were infected by the same pathogen at both sampling times (prevalence, 34.3%). Prevalence of IMI in the udder halves (36% (right) vs. 33% (left)) did not differ (P>0.05). All of the isolates were *Staphylococcus* spp. and the majority (66.7%) were *S. epidermidis*. Other species were *S. xylosus* (6.7%), *S. simulans* (5.5%), *S. caprae* (4.5%), and *S. hyicus* (3.3%). The remaining infections (3.3%) were caused by *S. hominis, S. aureus*, and *S. lugdunensis*. No identification was obtained for nine *Staphylococcus* spp. (10%). The *S. epidermidis* that was isolated showed a weak hemolysis at 48 h; and 73.3% had the same biochemical profile: positive reactions to fermentation of D-glucose, D-fructose, D-mannose, maltose, lactose, saccharose, and *n*-acetyl–glucosamine (weak); production of alkaline phosphatase; and production of acetyl-methyl–carbinol, arginine dehydrolase, and urease. All of the other substrates yielded negative reactions (Table 2).

Table 2
Biochemical characterization of the 60 *Staphylococcus epidermidis* identified strains

Reaction tested	+	−	±
D-Glucose fermentation	100	0	0
D-Fructose fermentation	100	0	0
D-Mannose fermentation	100	0	0
Maltose fermentation	0	100	0
D-Threolose fermentation	0	100	0
D-Mannitol fermentation	0	100	0
Xylitol fermentation	0	100	0
D-Melibiose fermentation	0	100	0
Reduction of nitrate to nitrite	26.6	73.3	0
Alkaline phosphatase	100	0	0
Acetyl-methyl–carbinol production	100	0	0
Raffinose fermentation	0	100	0
D-Xylose fermentation	0	100	0
Saccharose fermentation	100	0	0
α-methyl-D-glucoside fermentation	0	98.3	1.6
n-Acetyl–glusosamine fermentation	0	18.3	81.6
Arginine dehydrolase	93.3	6.6	0
Urease	93.3	1.6	5

Percentages of reactions that were positive (+), negative (−), or weak positive (±) after 24 h of incubation at 37°C.

Table 3
Geometric mean of SCC ($\times 10^3$/ml) for both sampling times and for both udder halves

Milk sample	1st sampling		2nd sampling	
Composite	1011[a]		1272[a]	
Foremilk	Udder half			
	Right	Left	Right	Left
Mean	985[a]	937[a]	1329[a]	1265[a]
Somatic cells ($\times 10^3$/ml) %				
<1000	54.1	46.7	34.4	31.9
1000–1999	22.2	30.9	35.1	27.2
>2000	23.7	22.6	30.5	31

[a]SCC within a row with no common superscript differ ($P<0.05$).

Of the 88 goat sera analyzed for CAEV antibodies, 65 were positive, 18 were weak positive, and 5 were negative.

Means of composite DHIA SCC were $>10^6$/ml for both samplings ($P>0.05$) (Table 3). When infection status was not considered, foremilk SCC ranged from 937 to 1329×10^3/ml. In the second sampling, foremilk SCC tended to be higher ($P>0.05$) than that for the first sampling. When infection status was considered, an increase in SCC was observed for infected glands, and this increase was higher for *S. epidermidis*. These differences were significant ($P<0.01$) only for infection status in the left halves of the udders (Table 4). Milk SCC increased ($P<0.01$) as parity increased (Table 4).

Table 4
Effect of infection status and parity on geometric mean foremilk SCC$\times 10^3$/ml

Infection status	Udder half	
	Right	Left
Uninfected	1297[a]	1014[a]
Staphylococcus epidermidis	1873[a]	1807[b]
Other staphylococci	1599[a]	1506[c]
Parity		
1	994[a]	891[a]
2	1706[b]	1397[b]
≥3	2281[c]	2213[c]

a,b,c: SCC within a column with no common superscripts differ ($P<0.01$).

4. Discussion

A wide range in the prevalence of subclinical IMI in goats has been reported. In data from 18 studies that reported IMI in goats (Contreras et al., 1995), eight studies showed similar or higher prevalences when compared with that of the present study (34.3%). However, most of the herds in those studies did not perform mastitis control procedures, such as teat dipping and antibiotic treatment at drying-off, to the extent practiced in the herd in this study. Unfortunately, not all researchers described the mastitis control procedures being used in the herds. When goat herds practiced similar mastitis control procedures as those practiced with the herd in this report, the prevalence rates were lower: 21% (Sánchez et al., 1997) and 6.5% (Contreras et al., 1997). Goat herds that did not practice teat dipping reported prevalences of 15.7% (Poutrel, 1984), 18% (Contreras et al., 1995), 33.4% (Lerondelle and Poutrel, 1984) and 65% (Kalidigrou-Vassiliadou, 1991).

In most studies of subclinical IMI in goats (Contreras et al., 1995; Deinhofer and Pernthaner, 1995; Lerondelle and Poutrel, 1984; Poutrel, 1984), CNS were the most frequently isolated organism; but our study is the first report claiming such a high rate (66.7% of all organisms isolated) belonging to a single species (*S. epidermidis*). Among CNS species isolated in goats, there are a few species that are frequently found, such as *S. caprae* (16–22%), *S. simulans* (9-12%), *S. chromogenes* (1–12%) and *S. xylosus* (1–6%) goats (Contreras et al., 1995; Deinhofer and Pernthaner, 1995; Poutrel, 1984).

Staphylococcus epidermidis has been described as a potential pathogen for the goat mammary gland; either increasing SCC or causing persistent infections. Our data show that left udder halves infected by *S. epidermidis* had significantly higher SCC than did either uninfected halves or halves infected by staphylococci other than *Staph. epidermidis*, but this effect was not found for right udder halves (Table 4). The increase of SCC agrees with the results of Deinhofer and Pernthaner (1995) who found a significant increase in SCC that accompanied caprine subclinical IMI caused by *S. epidermidis*. In addition, it has been demonstrated that *S. epidermidis* caused persistent IMI throughout lactation (Contreras et al., 1997). Previous records of the herd in this study

suggested that these pathogens were persisted since 1988.

Very little information concerning the sensitivity of *S. epidermidis* and other *Staphylococcus* spp. to teat dip is available. Teat dipping appears to influence the prevalence of *Staphylococcus* spp. (Harmon and Langlois, 1995). Langlois et al. (1984) suggested that germicidal teat dipping might alter the biochemical pattern of *Staphylococcus* spp. Hogan et al. (1987) found that *S. epidermidis* was the predominant staphylococci for lactating dairy cows when teat dipping was not practiced (37.1%). Furthermore, the distribution of *S. epidermidis* (22%) in herds that practiced teat dipping with linear dodecyl benzene sulfonic acid was more similar to the distribution of *S. epidermidis* in undipped herds than to the distribution of the pathogen in herds that practiced teat dipping with chlorhexidine (12.5%) or iodine (0%). This result was reflected by the high percentage of *S. epidermidis* found by Poutrel (1984) in goat herds that did not practice teat dipping (47.7%) and by the low percentage (4.6%) of *S. epidermidis* found in goat herds that used teat dips based on iodine (Contreras et al., 1997), which might explain the high prevalence of *S. epidermidis* in the herd in this study, because the postmilking teat dip was a disinfectant other than chlorhexidine or iodine.

Previous reports (Ryan et al., 1993; Smith and Cutlip, 1988) indicated a significant association between goats that was seropositive for CAEV and IMI caused by CNS though more recently, Nord (1997) did not find this association. The CAEV status of this herd might help explain the high prevalence of IMI caused by CNS, despite the mastitis control procedures used in the herd. In addition, Ryan et al. (1993) found in halves free of IMI that seropositive goats had a significantly greater mean SCC than seronegative does. These authors suggested that CAEV infection increases SCC similarly to IMI caused by CNS. A survey of the prevalence of the CAEV antibody in 196 dairy goat herds in 28 states indicated a prevalence of 31% (Cutlip et al., 1992). This situation complicates mastitis control measures that are based on SCC because does that are positive for CAEV can have high SCC in the absence of bacterial IMI (Ryan et al., 1993).

The high prevalence of IMI in the present study cannot explain the high SCC in uninfected udder halves ($1–1.3\times10^6$/ml). Factors unrelated to infection, such as parity and day of lactation, may contribute to increased milk SCC in goats (Dulin et al., 1983; Lerondelle et al., 1992; Wilson et al., 1995). Our study was conducted in October and November, and an increase in SCC in late lactation (fall and winter) has been reported by Wilson et al. (1995). Our data showed an effect of parity on SCC, and although the effect of day of lactation on SCC was not significant, the goats studied were in late lactation (mean=278 days of lactation).

5. Conclusions

A high prevalence of IMI caused by *S. epidermidis* was detected in a goat herd with previous records of high SCC. Bacterial infection increased SCC, but other factors, such as parity and possibly infection caused by CAEV, might have been involved. The high prevalence of CAEV might have predisposed does to an unusually high rate of IMI caused by *S. epidermidis*. Further studies are needed to evaluate the relationship between caprine arthritis–encephalitis virus infection and subclinical IMI and SCC.

Acknowledgements

The authors thank Brentland Farm and Goat Dairy (La Plata, MD) for making available the goats used in this study and the Animal Health Diagnostic Laboratory of Maryland for providing previous records of the herd studied. The senior author thanks the Dirección General de Investigación Científica y Técnica (Ministerio de Educación y Ciencia, Spain) for sponsoring his stay in the US.

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